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(54) Title: HEPATOCYTES AND CHONDROCYTES FROM ADHERENT PLACENTAL STEM CELLS; AND CD34+, CD45- PLACENTAL STEM CELL-ENRICHED CELL POPULATIONS

(57) Abstract: Provided herein are methods and compositions for the production of hepatocytes from placenta stem cells. Further provided herein is the use of such hepatocytes in the treatment of, and intervention in, for example, trauma, inflammation, and degenerative disorders of the liver. Also provided herein are compositions and methods relating to combinations of nanofibrous scaffolds and adherent placental stem cells and methods of using the same in cartilage repair. Finally, provided herein are compositions and methods relating to nonadherent, CD34<sup>+</sup>CD45<sup>+</sup> stem cells from placenta.

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**HEPATOCYTES AND CHONDROCYTES  
FROM ADHERENT PLACENTAL STEM CELLS; AND CD34<sup>+</sup>, CD45<sup>-</sup>  
PLACENTAL STEM CELL-ENRICHED CELL POPULATIONS**

[0001] This application claims benefit of U.S. Provisional Application No. 60/901,066, filed February 12, 2007; U.S. Provisional Application No. 60/901,076, filed February 12, 2007; U.S. Provisional Application No. 60/905,664, filed March 7, 2007; U.S. Provisional Application No. 60/906,064, filed March 8, 2007; and U.S. Provisional Application No. 60/966,577, filed August 28, 2007, the disclosures of each of which are incorporated herein in its entirety.

**1. FIELD**

[0002] Provided herein are methods and compositions relating to stem cells from placenta. Provided herein are methods for the production of hepatocytes from human adherent placental stem cells, and the use of such hepatocytes in the treatment of, and intervention in, for example, trauma, inflammatory and degenerative disorders of the liver. Also provided herein are compositions and methods relating to combinations of nanofibrous scaffolds and adherent placental stem cells and methods of using the same in cartilage repair. Finally, provided herein are compositions and methods relating to nonadherent, CD34<sup>+</sup>CD45<sup>-</sup> stem cells from placenta.

**2. BACKGROUND**

[0003] Somatic stem cells have been proposed for various therapeutic applications, including, for example, in animal models of cell replenishment therapy. The therapeutic potential of grafted stem cells can only be translated to clinical use if an ethically acceptable source of autologous stem cells is available, and if control of self renewal and fate decisions that program stem cell maturation into specific cell types is achieved.

[0004] A number of studies have described differentiation of embryonic stem cells down the hepatocyte lineage (*see, e.g.,* Sharma, N.S. *et al., Biotechnology & Bioengineering*, 94 (6): 1053- 93 (2006); Maguire, T., *et al, Biotechnology & Bioengineering*, 93(3):581-591(2006) and Chen Y, *et al., Cell Transplant.* 2006;15(10):865-71). In addition, human bone marrow derived mesenchymal cells were examined for the capacity to differentiate into functioning hepatocytes with some success (Ong SY, Dai H, Leong KW, *Tissue Eng.* 2006 Oct 1; Ong SY, Dai H, Leong KW *Biomaterials* (22):4087-97 (2006)(epub April 17, 2006); Sato Y, Araki H, Kato J, Nakamura K, *Blood.* 106(2):756-63 (2005) (epub April 7, 2005).

[0005] Hepatic disorders increasingly account for significant morbidity and mortality. Destruction of liver function by environmental and pathogenic causes presents significant

public health risks to otherwise healthy individuals. Replacement of damaged or killed hepatocytes in such damaged organs is therefore a significant clinical goal. However, an ethically acceptable source for stem cells that can differentiate into hepatocytes remains unavailable. These and other unmet needs are provided herein.

### 3. SUMMARY

[0006] In one aspect, provided herein are methods and compositions for the production of hepatocytes from adherent placental stem cells, and methods of using such hepatocytes to treat diseases, disorders or conditions, such as those involving trauma, inflammation, or systemic disorders of the liver, *e.g.*, diseases, disorders or conditions associated with hepatic inflammation. In one embodiment, provided herein is a method of producing a hepatocyte, comprising culturing a placental stem cell under conditions and for a time sufficient for said stem cell to exhibit a characteristic of a hepatocyte. In a specific embodiment, said characteristic is the production of albumin or expression of a gene encoding albumin. In another specific embodiment, said characteristic is the production of urea. In another specific embodiment, said culturing comprises contacting said stem cell with sodium butyrate. In another specific embodiment, said culturing comprises encapsulating said stem cell in alginate-poly-L-lysine. In another embodiment, provided herein is a hepatocyte produced by differentiation of a placenta-derived stem cell. Also provided herein is a method of treating a subject having a disease, disorder or condition associated with abnormal liver function, comprising introducing such a hepatocyte into said subject. In a more specific embodiment, the disease, disorder or condition is cirrhosis of the liver. In certain embodiments, the disease or conditions results from liver toxicity caused by, *e.g.*, alcohol or ingestion of toxins such as, *e.g.*, mushroom toxins. In certain embodiments, the disease or condition is a viral infection, *e.g.*, a hepatitis A, B, C, D, or E infection. In certain embodiments, the disease or condition is fulminant or subfulminant hepatitis. In another aspect, provided herein is a method for determining whether a compound has liver toxicity activity, comprising contacting a hepatocyte produced by differentiation of a placenta-derived stem cell with the compound, and determining whether the compound is toxic to the hepatocytes.

[0007] In another embodiment, the placental stem cell is positive for cytokeratin 18. In another embodiment, provided herein is a population of placental stem cells, or cells differentiated therefrom, at least 50%, 70%, 80%, 90%, 95% or 99% of which are positive for cytokeratin 18. In another embodiment, provided herein is a population of cells comprising placental stem cells, or cells differentiated therefrom, wherein at least 50%, 70%, 80%, 90%, 95% or 99% of the placental stem cells or cells differentiated therefrom are positive for

cytokeratin 18. In another embodiment, the invention provides a method of isolating a placental stem cell, or population of placental stem cells, or cells differentiated therefrom, comprising selecting a cytokeratin 18<sup>+</sup> placental stem cell, or cytokeratin 18<sup>+</sup> placental stem cells, and isolating said stem cell or stem cells from other placental cells.

[0008] In another aspect, provided herein is a composition comprising a plurality of cells encapsulated in alginate, wherein said cells are differentiated from placental stem cells. In one embodiment, said cells express at least one marker of a hepatocyte not expressed by, or expressed to a detectably different degree than, a placental stem cell. In another embodiment, said alginate is in the form of beads. In a specific embodiment, said beads are from about 200  $\mu\text{m}$  to about 800  $\mu\text{m}$  in size. In another specific embodiment, said beads average about 500  $\mu\text{m}$  in size.

[0009] In another aspect, provided herein is a mouse comprising human placental stem cell-derived hepatocytes or hepatogenic cells, wherein said mouse is produced by a method comprising the steps of: (a) irradiating said mouse with gamma radiation sufficient to kill substantially all of the endogenous bone marrow cells; (b) administering to said mouse sufficient bone marrow or bone marrow-derived cells from a NOD/SCID mouse to reconstitute the hematopoietic system of the mouse; and (c) transplanting to said mouse a plurality of hepatocytes or hepatogenic cells, wherein said hepatocytes or hepatogenic cells are differentiated from a plurality of CD10<sup>+</sup>, CD34<sup>+</sup>, CD105<sup>+</sup>, CD117<sup>+</sup>, CD200<sup>+</sup> placental stem cells. In one embodiment, said placental stem cell is additionally cytokeratin 18<sup>+</sup> and negative for at least one other cytokeratin expressed by differentiated hepatocytes. In another embodiment, said hepatocytes or hepatogenic cells are administered into an ear pinna of the mouse. In another embodiment, said hepatocytes or hepatogenic cells are infected with a virus. In a specific embodiment, said virus is hepatitis A virus, hepatitis B virus, hepatitis C virus, hepatitis D virus, or hepatitis E virus. In a more specific embodiment, the virus is hepatitis B virus.

[0010] In another aspect, provided herein is a method of identifying an antiviral agent, comprising contacting a mouse with a compound of interest, wherein serum from said mouse has detectable levels of virus, and wherein said compound is an antiviral agent if said contacting results in a detectable reduction in the amount of said virus in serum from said mouse, compared to serum from said mouse not contacted with the compound of interest, and wherein the mouse is produced by a method comprising the steps of: a. irradiating said mouse with gamma radiation sufficient to kill substantially all of the endogenous bone marrow cells; b. administering to said mouse sufficient bone marrow or bone marrow-derived

cells from a NOD/SCID mouse to reconstitute the hematopoietic system of the mouse; and c. transplanting to said mouse a plurality of hepatocytes or hepatogenic cells, wherein said hepatocytes or hepatogenic cells are differentiated from a plurality of CD10<sup>+</sup>, CD34<sup>+</sup>, CD105<sup>+</sup>, CD117<sup>+</sup>, CD200<sup>+</sup> placental stem cells. In one embodiment of the method, said virus is hepatitis A virus, hepatitis B virus, hepatitis C virus, hepatitis D virus, or hepatitis E virus. In specific embodiments of the method, an antigen or a nucleic acid of said virus is detected. In a more specific embodiment, said virus is hepatitis B virus. In a specific embodiment of the method, wherein a viral antigen is detected, said antigen is HBeAg or HBsAg. In another specific embodiment, wherein a viral nucleic acid is detected, said nucleic acid is the covalently closed circular form of hepatitis B virus. In a more specific embodiment, said nucleic acid is detected by PCR using primers specific for the covalently closed circular form of hepatitis B virus.

[0011] In another aspect, provided herein is a matrix, and compositions comprising such a matrix, wherein the matrix comprises placental stem cells that have differentiated to a hepatogenic or chondrogenic lineage, or to hepatocytes or chondrocytes. In a more specific embodiment, said matrix is a three-dimensional scaffold. In another more specific embodiment, said matrix comprises collagen, gelatin, laminin, fibronectin, pectin, ornithine, or vitronectin. In another more specific embodiment, said matrix is, or comprises, a nanofibrous scaffold, e.g., an electrospun nanofibrous scaffold. In a more specific embodiment, said nanofibrous scaffold comprises poly(L-lactic acid) (PLLA), type I collagen, a copolymer of vinylidene fluoride and trifluoroethylene (PVDF-TrFE), poly( $\epsilon$ -caprolactone), poly(L-lactide-co- $\epsilon$ -caprolactone) [P(LLA-CL)] (e.g., 75:25), and/or a copolymer of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) and type I collagen. In another more specific embodiment, said electrospun nanofibrous scaffold promotes the differentiation of placental stem cells into chondrocytes or hepatocytes. In another specific embodiment, the electrospun nanofibrous matrix or scaffold comprises placental stem cells that have differentiated into chondrocytic cells and/or chondrocytes, or into hepatocytic cells and/or hepatocytes. In another more specific embodiment, the matrix is an amniotic membrane or an amniotic membrane-derived biomaterial. In another more specific embodiment, said matrix comprises an extracellular membrane protein. In another more specific embodiment, said matrix comprises a synthetic compound. In another more specific embodiment, said matrix comprises a bioactive compound. In another more specific embodiment, said bioactive compound is a growth factor, cytokine, antibody, or organic molecule of less than 5,000 daltons.

[0012] In another embodiment, provided herein is a composition comprising isolated adherent CD10<sup>+</sup>, CD34<sup>+</sup>, CD105<sup>+</sup>, CD200<sup>+</sup> placental stem cells and an electrospun nanofibrous scaffold. In a specific embodiment, said nanofibrous scaffold comprises fibers of poly(L-lactic acid) (PLLA), poly lactic glycolic acid (PLGA), type I collagen, a copolymer of vinylidene fluoride and trifluoroethylene (PVDF-TrFE), poly( $\epsilon$ -caprolactone), poly(L-lactide-co- $\epsilon$ -caprolactone) [P(LLA-CL)] (e.g., 75:25), and/or a copolymer of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) and type I collagen. In another specific embodiment, said nanofibrous scaffold comprises fibers that average between about 250 nanometers and about 10  $\mu$ m in thickness. In another specific embodiment, said composition is contacted with conditions in which the placental stem cells differentiate into chondrogenic cells or chondrocytes. In another embodiment, provided herein is a method of making a composition comprising contacting adherent CD10<sup>+</sup>, CD34<sup>+</sup>, CD105<sup>+</sup>, CD200<sup>+</sup> placental stem cells with an electrospun nanofibrous scaffold, wherein said nanofibrous scaffold is made by electrospinning PLLA or PLGA at about 20 kV at about 30 cm needle to collector distance and about 0.05 mL/min. to about 0.1 mL/min flow rate, wherein said PLLA or PLGA are in solution at about 10% w/w to about 20% w/w.

[0013] In another aspect, provided herein is an isolated placental stem cell that is CD34<sup>+</sup> and CD45<sup>-</sup>. In a specific embodiment, said CD34<sup>+</sup>, CD45<sup>-</sup> stem cell is hematopoietic. In another specific embodiment, said CD34<sup>+</sup>, CD45<sup>-</sup> stem cell is non-adherent when cultured on a tissue culture surface, e.g., plastic. In a specific embodiment, provided herein is an isolated cell population enriched in placental stem cells that are CD34<sup>+</sup> and CD45<sup>-</sup>. In specific embodiments, at least 50%, 70%, 90% or 95% of cells in said population are CD34<sup>+</sup> CD45<sup>-</sup> placental stem cells. In another specific embodiment, the isolated cell population comprises proportionately more CD34<sup>+</sup> and CD45<sup>-</sup> placental stem cells than placental perfusate (e.g., perfusate from perfusion of a placenta with 750 mL 0.9% saline solution). In another specific embodiment, the isolated cell population comprises a stem cell that is not CD34<sup>+</sup> and CD45<sup>-</sup>. In a more specific embodiment, said stem cell that is not CD34<sup>+</sup> and CD45<sup>-</sup> is a CD34<sup>-</sup> adherent placental stem cell. In a more specific embodiment, said adherent placental stem cell is CD200<sup>+</sup>, CD105<sup>+</sup>, CD90<sup>+</sup>, CD10<sup>+</sup>, CD34<sup>-</sup> and/or CD45<sup>-</sup>. In another specific embodiment, said stem cell that is not CD34<sup>+</sup> and CD45<sup>-</sup> is a bone marrow-derived mesenchymal stem cell. In another specific embodiment, said stem cell that is not CD34<sup>+</sup> and CD45<sup>-</sup> is a CD34<sup>+</sup>, CD45<sup>+</sup> hematopoietic stem cell. In another specific embodiment, said stem cell that is not CD34<sup>+</sup> and CD45<sup>-</sup> is contained within cord blood or placental blood.

[0014] In another specific embodiment, the isolated cell population is a plurality of total nucleated cells (TNC) from placental perfusate. In a specific embodiment, the TNC from placental perfusate comprises placental cells from at least, or at most, 50, 100, 150, 200, 250, 300, 350, 400, 450 or 500 mL placental perfusate. In another specific embodiment, the TNC from placental perfusate have been treated to remove at least one type of non-red blood cell.

[0015] In another embodiment, the CD34<sup>+</sup>, CD45<sup>-</sup> hematopoietic placental stem cells are fetal (non-maternal). In another embodiment, the CD34<sup>+</sup>, CD45<sup>-</sup> hematopoietic placental stem cells are maternal. In another embodiment, an isolated population of hematopoietic placental stem cells comprises CD34<sup>+</sup>, CD45<sup>-</sup> hematopoietic placental stem cells that are fetal (non-maternal). In another embodiment, an isolated population of hematopoietic placental stem cells comprises CD34<sup>+</sup>, CD45<sup>-</sup> hematopoietic placental stem cells that are maternal.

[0016] In another aspect, provided herein are methods of isolating CD34<sup>+</sup>, CD45<sup>-</sup> hematopoietic placental stem cells. In one embodiment, the invention provides a method of isolating a CD34<sup>+</sup>, CD45<sup>-</sup> placental stem cell population, comprising selecting CD34<sup>+</sup> cells from a population of placental cells to form an isolated population of CD34<sup>+</sup> placental cells, and removing from said population of CD34<sup>+</sup> placental cells CD45<sup>+</sup> cells, wherein a CD34<sup>+</sup>, CD45<sup>-</sup> placental stem cell population is produced. In a specific embodiment, said selecting CD34<sup>+</sup> cells is done by immunoseparation. In another specific embodiment, said removing CD45<sup>+</sup> cells is done by immunoseparation. In another specific embodiment, said selecting or said removing is done by flow cytometry.

[0017] In another aspect, provided herein is a method of supplementing a cell population comprising adding a plurality of CD34<sup>+</sup>, CD45<sup>-</sup> hematopoietic placental stem cells to create a supplemented cell population, such that the supplemented cell population comprises substantially more CD34<sup>+</sup>, CD45<sup>-</sup> cells than before said supplementing. In various specific embodiments in this context, "substantially more" means at least 1, 2, 3, 4, 5, 6, 7, 8, 9 or at least 10% more. In other specific embodiments, the cell population to be supplemented comprises cord blood, placental blood, peripheral blood, or a combination thereof. In more specific embodiments, the cell population to be supplemented is cord blood, placental blood, peripheral blood, or a combination thereof. In another more specific embodiment, the cell population to be supplemented comprises nucleated cells isolated from cord blood, placental blood, peripheral blood, or a combination thereof. In other specific embodiments, the stem cell population to be supplemented comprises a population of hematopoietic stem cells, a population of adult stem cells, or a population of embryonic stem cells.

[0018] As used herein, the term "SH2" refers to an antibody that binds an epitope on the marker CD105. Thus, cells that are referred to as SH2<sup>+</sup> are CD105<sup>+</sup>.

[0019] As used herein, the terms "SH3" and SH4" refer to antibodies that bind epitopes present on the marker CD73. Thus, cells that are referred to as SH3<sup>+</sup> and/or SH4<sup>+</sup> are CD73<sup>+</sup>.

[0020] As used herein, the term "isolated stem cell" means a stem cell that is substantially separated from other, non-stem cells of the tissue, e.g., placenta, from which the stem cell is derived. A stem cell is "isolated" if at least 50%, 60%, 70%, 80%, 90%, 95%, or at least 99% of the non-stem cells with which the stem cell is naturally associated, or stem cells displaying a different marker profile, are removed from the stem cell, e.g., during collection and/or culture of the stem cell.

[0021] As used herein, the term "population of isolated cells" means a population of cells that is substantially separated from other cells of the tissue, e.g., placenta, from which the population of cells is derived. A stem cell is "isolated" if at least 50%, 60%, 70%, 80%, 90%, 95%, or at least 99% of the cells with which the population of cells, or cells from which the population of cells is derived, is naturally associated, i.e., stem cells displaying a different marker profile, are removed from the stem cell, e.g., during collection and/or culture of the stem cell.

[0022] As used herein, the term "placental stem cell" refers to a stem cell or progenitor cell, e.g., a multipotent cell, that is derived from a mammalian placenta, regardless of morphology, cell surface markers, or the number of passages after a primary culture. The term "placental stem cell" as used herein does not, however, refer to a trophoblast, cytotrophoblast, embryonic germ cell or embryonic stem cell. A cell is considered a "stem cell" if the cell retains at least one attribute of a stem cell, e.g., a marker or gene expression profile associated with one or more types of stem cells; the ability to replicate at least 10-40 times in culture; multipotency, e.g., the ability to differentiate, either *in vitro*, *in vivo* or both, into cells of one or more of the three germ layers; the lack of adult (i.e., differentiated) cell characteristics, or the like. The terms "placental stem cell" and "placenta-derived stem cell" may be used interchangeably. Unless otherwise noted herein, the term "placental" includes the umbilical cord. The adherent placental stem cells disclosed herein are, in certain embodiments, multipotent *in vitro* (that is, the cells differentiate *in vitro* under differentiating conditions), multipotent *in vivo* (that is, the cells differentiate *in vivo*), or both.

[0023] As used herein, a stem cell is "positive" for a particular marker when that marker is detectable above background. For example, a placental stem cell is positive for, e.g., CD73 because CD73 is detectable on placental stem cells, e.g., by flow cytometry, in an amount



detectably greater than background (in comparison to, *e.g.*, an isotype control). A cell is also positive for a marker when that marker can be used to distinguish the cell from at least one other cell type, or can be used to select or isolate the cell when present or expressed by the cell. In the context of, *e.g.*, antibody-mediated detection, "positive," as an indication a particular cell surface marker is present, means that the marker is detectable using an antibody, *e.g.*, a fluorescently-labeled antibody, specific for that marker; "positive" also means that a cell bears that marker in a amount that produces a signal, *e.g.*, in a cytometer, that is detectably above background. For example, a cell is "CD200<sup>+</sup>" where the cell is detectably labeled with an antibody specific to CD200, and the signal from the antibody is detectably higher than a control (*e.g.*, background). Conversely, "negative" in the same context means that the cell surface marker is not detectable using an antibody specific for that marker compared to background. For example, a cell is "CD34<sup>-</sup>" where the cell is not detectably labeled with an antibody specific to CD34. Unless otherwise noted herein, cluster of differentiation ("CD") markers are detected using antibodies. OCT-4 is determined to be present, and a cell is "OCT-4<sup>+</sup>" if OCT-4 is detectable using RT-PCR.

[0024] As used herein, "isolating" placental stem cells, *e.g.*, adherent placental stem cells or CD34<sup>+</sup>, CD45<sup>-</sup> stem cells, means to remove at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or 99% of the cells with which the stem cells are normally associated in the intact mammalian placenta. A stem cell from an organ is "isolated" when it is present in a population of cells that comprises fewer than 50% of the cells with which the stem cell is normally associated in the intact organ. "Dim", when associated with a cell marker, indicates that the marker is present detectably above background, but within about 5% to about 10% above background.

[0025] As used herein, "hepatocyte" means a cell that appears visually, biochemically and/or by gene expression pattern to be a hepatocyte as that term is normally understood. As used herein, "hepatogenic cell," referring to a cell differentiated from a placental stem cell or umbilical cord stem cells, is a cell that displays one or more characteristics of a terminally-differentiated hepatocyte, which characteristics are not found in a placental stem cell or umbilical cord stem cells, or are not found at the same level in a placental stem cell or umbilical cord stem cell (*e.g.*, are detectably higher or lower in a hepatogenic cell when compared to a placental stem cell or umbilical stem cell assayed for the characteristic under equivalent conditions), prior to differentiation into a hepatocyte or hepatogenic cell (*e.g.*, a placental stem cell or umbilical cord stem cell in an expansion culture). Thus, the various compositions, methods, and other embodiments of the present application also encompass

cells derived from placental stem cells that have fully or partially differentiated into hepatocytes.

#### 4. BRIEF DESCRIPTION OF THE DRAWINGS

[0026] FIG. 1: Viability of placental stem cells from perfusion (A), amnion (B), chorion (C), or amnion-chorion plate (D), or umbilical cord stem cells (E). Numbers on X-axis designate placenta from which stem cells were obtained.

[0027] FIG. 2: Percent HLA ABC<sup>+</sup>/CD45<sup>+</sup>/CD34<sup>+</sup>/CD133<sup>+</sup> cells from perfusion (A), amnion (B), chorion (C), or amnion-chorion plate (D), or umbilical cord stem cells (E) as determined by FACSCalibur. Numbers on X-axis designate placenta from which stem cells were obtained.

[0028] FIG. 3: Percent HLA ABC<sup>+</sup>/CD45<sup>+</sup>/CD34<sup>+</sup>/CD133<sup>+</sup> cells from perfusion (A), amnion (B), chorion (C), or amnion-chorion plate (D), or umbilical cord stem cells (E), as determined by FACS Aria. Numbers on X-axis designate placenta from which stem cells were obtained.

[0029] FIG. 4: HLA-G, CD10, CD13, CD33, CD38, CD44, CD90, CD105, CD117, CD200 expression in stem cells derived from placental perfusate.

[0030] FIG. 5: HLA-G, CD10, CD13, CD33, CD38, CD44, CD90, CD105, CD117, CD200 expression in stem cells derived from amnion.

[0031] FIG. 6: HLA-G, CD10, CD13, CD33, CD38, CD44, CD90, CD105, CD117, CD200 expression in stem cells derived from chorion.

[0032] FIG. 7: HLA-G, CD10, CD13, CD33, CD38, CD44, CD90, CD105, CD117, CD200 expression in stem cells derived from amnion-chorion plate.

[0033] FIG. 8: HLA-G, CD10, CD13, CD33, CD38, CD44, CD90, CD105, CD117, CD200 expression in stem cells derived from umbilical cord.

[0034] FIG. 9: Average expression of HLA-G, CD10, CD13, CD33, CD38, CD44, CD90, CD105, CD117, CD200 expression in stem cells derived from perfusion (A), amnion (B), chorion (C), amnion-chorion plate (D) or umbilical cord (E).

[0035] FIG. 10: Average percentage of total cells from six matched human placental perfusate and umbilical cord units. X axis: percent cells, of total nucleated cells, of the phenotype shown on the Y-axis.

## 5. DETAILED DESCRIPTION

### 5.1 Production of Hepatocytes

[0036] In the sections and discussions that follow, it will be understood by the skilled artisan that many of the various compositions and methods can be performed on or using placental stem cells or umbilical cord stem cells that have differentiated, or have been differentiated, down the hepatocyte lineage.

[0037] In one aspect, provided herein are methods and compositions for the production of hepatocytes and/or hepatogenic cells from placenta-derived cells, particularly placental stem cells or umbilical cord stem cells. As used herein, "placental stem cells" or "umbilical cord stem cells" means adherent stem cells unless otherwise specified. Stem cells may be obtained from a mammalian placenta or umbilical cord by perfusion (*see, e.g.,* Hariri, U.S. Patent Nos. 7,045,148 and 7,255,879, which are hereby incorporated herein in their entireties. Stem cells may also be obtained from placenta or umbilical cord by disruption (*e.g.,* maceration) of a placenta or part thereof (*see, e.g.,* Section 6.2, below). Cells displaying hepatocyte characteristics, *e.g.,* hepatocytes and/or hepatogenic cells, may be obtained from placental stem cells. These cells are useful in the treatment of diseases, disorders or conditions associated with, for example, cirrhosis of the liver, including, but not limited to cirrhosis caused by alcohol ingestion, ingestion of hepatic toxins such as those found in, *e.g.,* mushrooms of the genus *Amanita*, or caused by viral infections, *e.g.,* hepatitis A, B, C, D, or E infection.

[0038] In one embodiment, differentiable cells, such as stem cells, may be obtained from the placenta or umbilical cord as follows. Primary cultures of mononuclear cells (MNCs) are isolated from placentas, *e.g.,* from human placenta perfusates or from physically and/or enzymatically-disrupted placental tissue. The placentas are obtained following birth of full-term infants under informed consent of the donors. Briefly, for perfusion, umbilical vessels are cannulated then connected to a flow-controlled circuit, and the placenta is perfused at, *e.g.,* 1 mL/min (room temperature, up to 24 hours) with Dulbecco's modified Eagle's medium (DMEM, Gibco/BRL) containing high glucose, 1% heparin and penicillin/streptomycin. Placenta perfusate (750 mL) is then pooled, centrifuged, and the cell pellet resuspended in PBS containing 1% fetal calf serum (FBS) then separated by differential gradient density centrifugation through Lymphoprep™ (Gibco/BRL). The buffy-coat interface containing mononucleated cells including adherent placental stem cells are recovered, resuspended in DMEM/10% FBS, plated on fibronectin-coated (Sigma) Falcon plates and incubated at 37°C with 5% humidified CO<sub>2</sub>. After a 24-hour incubation the

nonadherent cells are discarded and the adherent cells are maintained and expanded in fresh culture media; individual cell colonies develop between 10 and 18 days and are expanded as placental stem cell lines.

[0039] Human adherent placental stem cells display fibroblast-like morphology in culture and are HLA-class I positive. Using FACS analysis these cells do not express the hematopoietic markers CD34 or CD45. However, they do express the multipotential cellular markers CD10 (CALLA), CD29 ( $\beta_1$  integrin), CD54 (ICAM-1), CD90 (Thy-1) as well as SH2 (CD105), SH3 (CD73) and CD200. Under standard growth conditions the doubling time for placental stem cells is about 18 to 36 hours, and the cells maintain this phenotype for greater than 40 population doublings in vitro. Human adherent placental stem cells are distinguishable from human embryonic stem cells or embryonic germ cells in that human embryonic stem cells or germ cells are obtained only from the inner cell mass of the blastula or fetal gonads, not placentas. Human adherent placental stem cells are also distinguishable from mesenchymal stem cells from, *e.g.*, bone marrow, cord blood or peripheral blood, or bone marrow-derived stem cells, in that placental stem cells form embryoid-like bodies in culture, while mesenchymal stem cells or bone marrow-derived stem cells do not, and placental stem cells display unique gene expression pattern relative to mesenchymal stem cells. *See* U.S. Patent Application No. 11/648,813, filed December 28, 2006, the disclosure of which is hereby incorporated herein by reference in its entirety.

[0040] Placental stem cells may be differentiated to hepatocytes by culturing in culture medium comprising sodium butyrate or by encapsulating the cells in a suitable microcapsule polymer, *e.g.* alginate-poly-L-lysine. Hepatocytes can be produced from placenta-derived stem cells as described above, and maintained or cultured as described in below. Hepatocyte differentiation can be assessed using flow cytometry and monitoring for particular gene expression or enzymatic activity as described below.

## 5.2 Placental Stem Cells and Placental Stem Cell Populations

[0041] In one aspect, the methods provided herein use adherent placental stem cells, that is, stem cells obtainable from a placenta or part thereof, *e.g.*, amnion, chorion, amnion/chorion plate, umbilical cord, *etc.*, that (1) adhere to a tissue culture substrate; and (2) differentiate into one or more non-placental cell types, and/or cells having tissue-specific cell characteristics, under the appropriate differentiation conditions. Placental stem cells are not derived from, nor are they derivable from, blood, *e.g.*, placental blood or umbilical cord blood, or from bone marrow.

[0042] Placental stem cells can be either fetal or maternal in origin (that is, can have the genotype of either the mother or fetus). Populations of placental stem cells, or populations of cells comprising placental stem cells, can comprise placental stem cells that are solely fetal or maternal in origin, or can comprise a mixed population of placental stem cells of both fetal and maternal origin. The placental stem cells, and populations of cells comprising the placental stem cells, can be identified and selected by the morphological, marker, and culture characteristics discussed below.

### 5.2.1 Physical and Morphological Characteristics

[0043] The placental stem cells used in the methods disclosed herein, when cultured in primary culture or in cell culture, adhere to the tissue culture substrate, *e.g.*, tissue culture container surface (*e.g.*, tissue culture plastic). Placental stem cells in culture, *e.g.*, on a tissue culture surface, assume a generally fibroblastoid appearance, with a number of cytoplasmic processes extending from the central cell body. The placental stem cells are, however, morphologically distinguishable from fibroblasts cultured under the same conditions, as the placental stem cells exhibit a greater number of such processes than do fibroblasts. Morphologically, placental stem cells are also distinguishable from hematopoietic stem cells, which generally assume a more rounded, or cobblestone, morphology in culture.

### 5.2.2 Cell Surface, Molecular and Genetic Markers

[0044] Adherent placental stem cells, and populations of adherent placental stem cells, useful in the methods and compositions described herein, express a plurality of markers that can be used to identify and/or isolate the stem cells, or populations of cells that comprise the stem cells. The placental stem cells, and stem cell populations (that is, two or more placental stem cells) described herein include stem cells and stem cell-containing cell populations obtained directly from the placenta, or any part thereof (*e.g.*, amnion, chorion, placental cotyledons, umbilical cord, and the like). Placental stem cell populations also includes populations of (that is, two or more) placental stem cells in culture, and a population in a container, *e.g.*, a bag. Placental stem cells are not, however, trophoblasts.

[0045] The placental stem cells described herein are multipotent in that they can be differentiated *in vitro* into cells representative of the three germ layers, *e.g.*, adipocytic cells, chondrocytic cells, hepatic cells, neurogenic cells, cardiac cells, and the like. The placental stem cells described herein, however, need not differentiate *in vivo* to be considered multipotent, or to be useful. The term "placental stem cell," therefore, encompasses cells described herein that differentiate *in vitro* but not *in vivo*, differentiate *in vivo* but not *in vitro*,

or both *in vitro* and *in vivo*. In one embodiment, the placental stem cells provided herein can be differentiated *in vitro* into cells representative of one or more of the three germ layers, but do not differentiate *in vivo*, *e.g.*, in a NOD-SCID mouse.

[0046] Adherent (non-hematopoietic) placental stem cells generally express the markers CD73, CD105, CD200, HLA-G, and/or OCT-4, and do not express CD34, CD38, or CD45. Placental stem cells can also express HLA-ABC (MHC-I), but generally do not express HLA-DR. In a specific embodiment, adherent placental stem cells are CD10<sup>+</sup>, CD34<sup>-</sup>, CD105<sup>+</sup> and CD200<sup>+</sup>. These markers can be used to identify placental stem cells, and to distinguish placental stem cells from other stem cell types. Because the placental stem cells can express CD73 and CD105, they can have mesenchymal stem cell-like characteristics. However, because the placental stem cells can express CD200 and HLA-G, a fetal-specific marker, they can be distinguished from mesenchymal stem cells, *e.g.*, bone marrow-derived mesenchymal stem cells, which express neither CD200 nor HLA-G. In the same manner, the lack of expression of CD34, CD38 and/or CD45 identifies the placental stem cells as non-hematopoietic stem cells. Such placental stem cells, and populations of cells comprising such placental stem cells, can be differentiated into hepatocytes, hepatogenic cells, populations of hepatocytes, populations of hepatogenic cells, and combinations of the foregoing.

[0047] In one embodiment, the methods and compositions provided herein use an isolated placental stem cell that is CD200<sup>+</sup> and HLA-G<sup>+</sup>. In specific embodiments, said stem cell is also CD73<sup>+</sup> and CD105<sup>+</sup>. In another specific embodiment, said stem cell is also CD34<sup>-</sup>, CD38<sup>-</sup> or CD45<sup>-</sup>. In a more specific embodiment, said stem cell is also CD34<sup>-</sup>, CD38<sup>-</sup>, CD45<sup>-</sup>, CD73<sup>+</sup> and CD105<sup>+</sup>. In another specific embodiment, said stem cell has been expanded, for example, passaged at least once, at least three times, at least five times, at least 10 times, at least 15 times, or at least 20 times.

[0048] In another embodiment, the methods and compositions provided herein use an isolated cell population comprising a plurality of placental stem cells that are CD200<sup>+</sup>, HLA-G<sup>+</sup>. In various embodiments, at least 10%, at least 20%, at least 30%, at least 40%, at least 50% at least 60%, at least 70%, at least 80%, at least 90%, or at least 95% of said placental stem cells in said population are said CD200<sup>+</sup>, HLA-G<sup>+</sup> stem cells. In a specific embodiment of the isolated populations, said stem cells are also CD73<sup>+</sup> and CD105<sup>+</sup>. In another specific embodiment, said stem cells are also CD34<sup>-</sup>, CD38<sup>-</sup> or CD45<sup>-</sup>. In a more specific embodiment, said stem cells are also CD34<sup>-</sup>, CD38<sup>-</sup>, CD45<sup>-</sup>, CD73<sup>+</sup> and CD105<sup>+</sup>. In another embodiment, said isolated population produces one or more embryoid-like bodies when cultured under conditions that allow the formation of embryoid-like bodies. In another

specific embodiment, said population has been expanded, for example, passaged at least once, at least three times, at least five times, at least 10 times, at least 15 times, or at least 20 times.

**[0049]** In another embodiment, the methods and compositions provided herein use an isolated placental stem cell that is CD73<sup>+</sup>, CD105<sup>+</sup>, CD200<sup>+</sup>. In a specific embodiment of said populations, said stem cell is also HLA-G<sup>+</sup>. In another specific embodiment, said stem cell is also CD34<sup>-</sup>, CD38<sup>-</sup> or CD45<sup>-</sup>. In another specific embodiment, said stem cell is also CD34<sup>-</sup>, CD38<sup>-</sup> and CD45<sup>-</sup>. In a more specific embodiment, said stem cell is also CD34<sup>-</sup>, CD38<sup>-</sup>, CD45<sup>-</sup>, and HLA-G<sup>+</sup>. In another specific embodiment, said stem cell has been expanded, for example, passaged at least once, at least three times, at least five times, at least 10 times, at least 15 times, or at least 20 times.

**[0050]** In another embodiment, the methods and compositions provided herein use an isolated cell population comprising a plurality of placental stem cells that are CD73<sup>+</sup>, CD105<sup>+</sup>, CD200<sup>+</sup>. In various embodiments, at least 10%, at least 20%, at least 30%, at least 40%, at least 50% at least 60%, at least 70%, at least 80%, at least 90%, or at least 95% of said placental stem cells in said population are said CD73<sup>+</sup>, CD105<sup>+</sup>, CD200<sup>+</sup> cells. In a specific embodiment of said populations, said stem cells are HLA-G<sup>+</sup>. In another specific embodiment, said stem cells are CD34<sup>-</sup>, CD38<sup>-</sup> or CD45<sup>-</sup>. In another specific embodiment, said stem cells are CD34<sup>-</sup>, CD38<sup>-</sup> and CD45<sup>-</sup>. In a more specific embodiment, said stem cells are CD34<sup>-</sup>, CD38<sup>-</sup>, CD45<sup>-</sup>, and HLA-G<sup>+</sup>. In another specific embodiment, said population of cells produces one or more embryoid-like bodies when cultured under conditions that allow the formation of embryoid-like bodies. In another specific embodiment, said population has been expanded, for example, passaged at least once, at least three times, at least five times, at least 10 times, at least 15 times, or at least 20 times.

**[0051]** In another embodiment, the methods and compositions provided herein use an isolated placental stem cell that is CD200<sup>+</sup>, OCT-4<sup>+</sup>. In a specific embodiment, said stem cell is also CD73<sup>+</sup> and CD105<sup>+</sup>. In another specific embodiment, said stem cell is also HLA-G<sup>+</sup>. In another specific embodiment, said stem cell is also CD34<sup>-</sup>, CD38<sup>-</sup> and CD45<sup>-</sup>. In a more specific embodiment, said stem cell is also CD34<sup>-</sup>, CD38<sup>-</sup>, CD45<sup>-</sup>, CD73<sup>+</sup>, CD105<sup>+</sup> and HLA-G<sup>+</sup>. In another specific embodiment, said stem cell has been expanded, for example, passaged at least once, at least three times, at least five times, at least 10 times, at least 15 times, or at least 20 times.

**[0052]** In another embodiment, the methods and compositions provided herein use an isolated cell population comprising a plurality of placental stem cells that are CD200<sup>+</sup>, OCT-

4<sup>+</sup>. In various embodiments, at least 10%, at least 20%, at least 30%, at least 40%, at least 50% at least 60%, at least 70%, at least 80%, at least 90%, or at least 95% of said placental stem cells in said population are said CD200<sup>+</sup>, OCT-4<sup>+</sup> cells. In a specific embodiment, said stem cells are CD73<sup>+</sup> and CD105<sup>+</sup>. In another specific embodiment, said stem cells are HLA-G<sup>+</sup>. In another specific embodiment, said stem cells are CD34<sup>-</sup>, CD38<sup>-</sup> and CD45<sup>-</sup>. In a more specific embodiment, said stem cells are CD34<sup>-</sup>, CD38<sup>-</sup>, CD45<sup>-</sup>, CD73<sup>+</sup>, CD105<sup>+</sup> and HLA-G<sup>+</sup>. In another specific embodiment, the population produces one or more embryoid-like bodies when cultured under conditions that allow the formation of embryoid-like bodies. In another specific embodiment, said population has been expanded, for example, passaged at least once, at least three times, at least five times, at least 10 times, at least 15 times, or at least 20 times.

[0053] In another embodiment, the methods and compositions provided herein use an isolated placental stem cell that is CD73<sup>+</sup>, CD105<sup>+</sup> and HLA-G<sup>+</sup>. In a specific embodiment of the above plurality, said stem cell is also CD34<sup>-</sup>, CD38<sup>-</sup> or CD45<sup>-</sup>. In another specific embodiment, said stem cell is also CD34<sup>-</sup>, CD38<sup>-</sup> and CD45<sup>-</sup>. In another specific embodiment, said stem cells are also OCT-4<sup>+</sup>. In another specific embodiment, said stem cell is also CD200<sup>+</sup>. In a more specific embodiment, said stem cell is also CD34<sup>-</sup>, CD38<sup>-</sup>, CD45<sup>-</sup>, OCT-4<sup>+</sup> and CD200<sup>+</sup>. In another specific embodiment, said stem cell has been expanded, for example, passaged at least once, at least three times, at least five times, at least 10 times, at least 15 times, or at least 20 times.

[0054] In another embodiment, the methods and compositions provided herein use an isolated cell population comprising a plurality of placental stem cells that are CD73<sup>+</sup>, CD105<sup>+</sup> and HLA-G<sup>+</sup>. In various embodiments, at least 10%, at least 20%, at least 30%, at least 40%, at least 50% at least 60%, at least 70%, at least 80%, at least 90%, or at least 95% of said placental stem cells in said population are said CD73<sup>+</sup>, CD105<sup>+</sup> and HLA-G<sup>+</sup> cells. In a specific embodiment of the above plurality, said stem cells are also CD34<sup>-</sup>, CD38<sup>-</sup> or CD45<sup>-</sup>. In another specific embodiment, said stem cells are also CD34<sup>-</sup>, CD38<sup>-</sup> and CD45<sup>-</sup>. In another specific embodiment, said stem cells are also OCT-4<sup>+</sup>. In another specific embodiment, said stem cells are also CD200<sup>+</sup>. In a more specific embodiment, said stem cells are also CD34<sup>-</sup>, CD38<sup>-</sup>, CD45<sup>-</sup>, OCT-4<sup>+</sup> and CD200<sup>+</sup>. In another specific embodiment, said population has been expanded, for example, passaged at least once, at least three times, at least five times, at least 10 times, at least 15 times, or at least 20 times.

[0055] In another embodiment, the methods and compositions provided herein use an isolated cell population comprising a plurality of placental stem cells that are CD73<sup>+</sup>, CD105<sup>+</sup>



stem cells, wherein said plurality forms one or more embryoid-like bodies under conditions that allow formation of embryoid-like bodies. In various embodiments, at least 10%, at least 20%, at least 30%, at least 40%, at least 50% at least 60%, at least 70%, at least 80%, at least 90%, or at least 95% of said placental stem cells in said population are said CD73<sup>+</sup>, CD105<sup>+</sup> stem cells. In a specific embodiment, said stem cells are also CD34<sup>+</sup>, CD38<sup>-</sup> or CD45<sup>-</sup>. In another specific embodiment, said stem cells are also CD34<sup>+</sup>, CD38<sup>-</sup> and CD45<sup>-</sup>. In another specific embodiment, said stem cells are also OCT-4<sup>+</sup>. In a more specific embodiment, said stem cells are also OCT-4<sup>+</sup>, CD34<sup>+</sup>, CD38<sup>-</sup> and CD45<sup>-</sup>. In another specific embodiment, said population has been expanded, for example, passaged at least once, at least three times, at least five times, at least 10 times, at least 15 times, or at least 20 times.

**[0056]** In another embodiment, the methods and compositions provided herein use an isolated cell population comprising a plurality of placental stem cells that are OCT-4<sup>+</sup> stem cells, wherein said population forms one or more embryoid-like bodies when cultured under conditions that allow the formation of embryoid-like bodies. In various embodiments, at least 10%, at least 20%, at least 30%, at least 40%, at least 50% at least 60%, at least 70%, at least 80%, at least 90%, or at least 95% of said placental cells in said population are said OCT4<sup>+</sup> stem cells. In a specific embodiment of the above populations, said stem cells are CD73<sup>+</sup> and CD105<sup>+</sup>. In another specific embodiment, said stem cells are CD34<sup>+</sup>, CD38<sup>-</sup>, or CD45<sup>-</sup>. In another specific embodiment, said stem cells are CD200<sup>+</sup>. In a more specific embodiment, said stem cells are CD73<sup>+</sup>, CD105<sup>+</sup>, CD200<sup>+</sup>, CD34<sup>+</sup>, CD38<sup>-</sup>, and CD45<sup>-</sup>. In another specific embodiment, said population has been expanded, for example, passaged at least once, at least three times, at least five times, at least 10 times, at least 15 times, or at least 20 times.

**[0057]** In another embodiment, the methods and compositions provided herein use an isolated placental stem cell that is, or a cell population comprising a plurality of placental stem cells that are, CD29<sup>+</sup>, CD44<sup>+</sup>, CD73<sup>+</sup>, CD90<sup>+</sup>, CD105<sup>+</sup>, CD200<sup>+</sup>, CD34<sup>+</sup> and CD133<sup>+</sup>.

**[0058]** In another embodiment, the methods and compositions provided herein use an isolated placental stem cell that is CD10<sup>+</sup>, CD34<sup>+</sup>, CD105<sup>+</sup>, and CD200<sup>+</sup>. Further provided herein is an isolated population of cells, *e.g.*, placental stem cells, wherein at least about 70%, at least about 80%, at least about 90%, at least about 95% or at least about 99% of said placental stem cells are CD10<sup>+</sup>, CD34<sup>+</sup>, CD105<sup>+</sup>, CD200<sup>+</sup>. In a specific embodiment of the above embodiments, said stem cells are additionally CD90<sup>+</sup> and CD45<sup>-</sup>. In a specific embodiment, said stem cell or population of placental stem cells is isolated away from placental cells that are not stem cells. In another specific embodiment, said stem cell or

population of placental stem cells is isolated away from placental stem cells that do not display these characteristics. In another specific embodiment, said isolated placental stem cell is non-maternal in origin. In another specific embodiment, at least about 90%, at least about 95%, or at least about 99% of said cells in said isolated population of placental stem cells, are non-maternal in origin.

[0059] In another embodiment, the methods and compositions provided herein use an isolated placental stem cell that is HLA-A,B,C<sup>-</sup>, CD45<sup>-</sup>, CD133<sup>-</sup> and CD34<sup>-</sup>. Further provided herein is the use of an isolated population of placental stem cells, wherein at least about 70%, at least about 80%, at least about 90%, at least about 95% or at least about 99% of said placental stem cells are HLA-A,B,C<sup>-</sup>, CD45<sup>-</sup>, CD133<sup>-</sup> and CD34<sup>-</sup>. In a specific embodiment, said stem cell or population of placental stem cells is isolated away from placental cells that are not stem cells. In another specific embodiment, said population of placental stem cells is isolated away from placental stem cells that do not display these characteristics. In another specific embodiment, said isolated placental stem cell is non-maternal in origin. In another specific embodiment, at least about 90%, at least about 95%, or at least about 99% of said cells in said isolated population of placental stem cells, are non-maternal in origin. In another embodiment, the HLA-A,B,C<sup>-</sup>, CD45<sup>-</sup>, CD133<sup>-</sup> and CD34<sup>-</sup> placental stem cell is a stem cell isolated from placental perfusate. In another embodiment, the HLA-A,B,C<sup>-</sup>, CD45<sup>-</sup>, CD133<sup>-</sup> and CD34<sup>-</sup> placental stem cell is a stem cell isolated by physical and/or enzymatic disruption of placental tissue.

[0060] In another embodiment, the methods and compositions provided herein an isolated placental stem cell that is CD10<sup>+</sup>, CD13<sup>+</sup>, CD33<sup>+</sup>, CD45<sup>-</sup>, CD117<sup>-</sup> and CD133<sup>-</sup>. Further provided herein is an isolated population of placental stem cells, wherein at least about 70%, at least about 80%, at least about 90%, at least about 95% or at least about 99% of said placental stem cells are CD10<sup>+</sup>, CD13<sup>+</sup>, CD33<sup>+</sup>, CD45<sup>-</sup>, CD117<sup>-</sup> and CD133<sup>-</sup>. In a specific embodiment, said stem cell or population of placental stem cells is isolated away from placental cells that are not stem cells. In another specific embodiment, said isolated placental stem cell is non-maternal in origin. In another specific embodiment, at least about 90%, at least about 95%, or at least about 99% of said cells in said isolated population of placental stem cells, are non-maternal in origin. In another specific embodiment, said stem cell or population of placental stem cells is isolated away from placental stem cells that do not display these characteristics. In another embodiment, provided herein is a method of obtaining a placental stem cell that is CD10<sup>+</sup>, CD13<sup>+</sup>, CD33<sup>+</sup>, CD45<sup>-</sup>, CD117<sup>-</sup> and CD133<sup>-</sup> comprising isolating said cell from placental perfusate. In another embodiment, the HLA-

A<sub>2</sub>B<sub>2</sub>C<sup>+</sup>, CD45<sup>+</sup>, CD133<sup>+</sup> and CD34<sup>+</sup> placental stem cell is a stem cell isolated by physical and/or enzymatic disruption of placental tissue.

[0061] In another embodiment, the methods and compositions provided herein an isolated placental stem cell that is CD10<sup>+</sup>, CD33<sup>+</sup>, CD44<sup>+</sup>, CD45<sup>+</sup>, and CD117<sup>+</sup>. Further provided herein is an isolated population of placental stem cells, wherein at least about 70%, at least about 80%, at least about 90%, at least about 95% or at least about 99% of said placental stem cells are CD10<sup>+</sup>, CD33<sup>+</sup>, CD44<sup>+</sup>, CD45<sup>+</sup>, and CD117<sup>+</sup>. In a specific embodiment, said stem cell or population of placental stem cells is isolated away from placental cells that are not stem cells. In another specific embodiment, said isolated placental stem cell is non-maternal in origin. In another specific embodiment, at least about 90%, at least about 95%, or at least 99% of said cells in said isolated population of placental stem cells, are non-maternal in origin. In another specific embodiment, said stem cell or population of placental stem cells is isolated away from placental stem cells that do not display these characteristics. In another embodiment, provided herein is a method of obtaining a placental stem cell that is CD10<sup>+</sup>, CD33<sup>+</sup>, CD44<sup>+</sup>, CD45<sup>+</sup>, CD117<sup>+</sup> comprising isolating said cell from placental perfusate. In another embodiment, the HLA-A,B,C<sup>+</sup>, CD45<sup>+</sup>, CD133<sup>+</sup> and CD34<sup>+</sup> placental stem cell is a stem cell isolated by physical and/or enzymatic disruption of placental tissue.

[0062] In another embodiment, the methods and compositions provided herein use an isolated placental stem cell that is CD10<sup>+</sup>, CD13<sup>+</sup>, CD33<sup>+</sup>, CD45<sup>+</sup>, and CD117<sup>+</sup>. Further provided herein an isolated population of placental stem cells, wherein at least about 70%, at least about 80%, at least about 90%, at least about 95% or at least about 99% of said placental stem cells are CD10<sup>+</sup>, CD13<sup>+</sup>, CD33<sup>+</sup>, CD45<sup>+</sup>, and CD117<sup>+</sup>. In a specific embodiment, said stem cell or population of placental stem cells is isolated away from placental cells that are not stem cells. In another specific embodiment, said isolated placental stem cell is non-maternal in origin. In another specific embodiment, at least about 90%, at least about 95%, or at least 99% of said cells in said isolated population of placental stem cells, are non-maternal in origin. In another specific embodiment, said stem cell or population of placental stem cells is isolated away from placental stem cells that do not display these characteristics. In another embodiment, provided herein is a method of obtaining a placental stem cell that is CD10<sup>+</sup>, CD13<sup>+</sup>, CD33<sup>+</sup>, CD45<sup>+</sup>, and CD117<sup>+</sup> comprising isolating said cell from placental perfusate. In another embodiment, the HLA-A,B,C<sup>+</sup>, CD45<sup>+</sup>, CD133<sup>+</sup> and CD34<sup>+</sup> placental stem cell is a stem cell isolated by physical and/or enzymatic disruption of placental tissue.

[0063] In another embodiment, the methods and compositions provided herein use an isolated placental stem cell that is HLA A,B,C<sup>+</sup>, CD45<sup>+</sup>, CD34<sup>+</sup>, CD133<sup>+</sup>, positive for CD10,

CD13, CD38, CD44, CD90, CD105, CD200 and/or HLA-G, and/or negative for CD117. In another embodiment, the isolated population of placental stem cells used in the methods and compositions provided herein are HLA A,B,C<sup>+</sup>, CD45<sup>+</sup>, CD34<sup>+</sup>, CD133<sup>+</sup>, and at least about 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or about 99% of the stem cells in the population are positive for CD10, CD13, CD38, CD44, CD90, CD105, CD200 and/or HLA-G, and/or negative for CD117. In a specific embodiment, said stem cell or population of placental stem cells is isolated away from placental cells that are not stem cells. In another specific embodiment, said isolated placental stem cell is non-maternal in origin. In another specific embodiment, at least about 90%, at least about 95%, or at least about 99%, of said cells in said isolated population of placental stem cells, are non-maternal in origin. In another specific embodiment, said stem cell or population of placental stem cells is isolated away from placental stem cells that do not display these characteristics. In another embodiment, provided herein is a method of obtaining a placental stem cell that is HLA A,B,C<sup>+</sup>, CD45<sup>+</sup>, CD34<sup>+</sup>, CD133<sup>+</sup> and positive for CD10, CD13, CD38, CD44, CD90, CD105, CD200 and/or HLA-G, and/or negative for CD117, comprising isolating said cell from placental perfusate.

[0064] In another embodiment, the methods and compositions provided herein use a placental stem cell that is CD200<sup>+</sup> and CD10<sup>+</sup>, as determined by antibody binding, and CD117<sup>+</sup>, as determined by both antibody binding and RT-PCR, or a population of such cells, or a population of cells comprising such isolated placental stem cells. In another embodiment, the methods and compositions provided herein use a placental stem cell that is CD10<sup>+</sup>, CD29<sup>+</sup>, CD54<sup>+</sup>, CD200<sup>+</sup>, HLA-G<sup>+</sup>, HLA class I<sup>+</sup> and  $\beta$ -2-microglobulin<sup>+</sup>. In another embodiment, provided herein are placental stem cells, wherein the expression of at least one marker is at least two-fold higher than for a mesenchymal stem cell (e.g., a bone marrow-derived mesenchymal stem cell). In another specific embodiment, said isolated placental stem cell is non-maternal in origin. In another specific embodiment, at least about 90%, at least about 95%, or at least 99%, of said cells in said isolated population of placental stem cells, are non-maternal in origin.

[0065] In another embodiment, placental stem cells used in the methods and compositions provided herein are positive for cytokeratin 18. In another embodiment, provided herein is a population of placental stem cells, or cells differentiated therefrom, at least 50%, 70%, 80%, 90%, 95% or 99% of which are positive for cytokeratin 18. In another embodiment, provided herein is a population of cells comprising placental stem cells, or cells differentiated therefrom, wherein at least 50%, 70%, 80%, 90%, 95% or 99% of the placental stem cells or